Report for 2001ME2261B: A Seed Grant for Determining the Risk of Exposure to Dioxin and PCBs in Natural Water

There are no reported publications resulting from this project.

Report Follows:

DETERMINATION OF LEVELS OF DIOXIN IN NATURAL WATER USING IMMUNOASSAY

A Report to Maine Water Research Institute Regarding Results Gathered From Seed Grant

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Abstract:

Activated carbon, C_{18}, and carbon nanotubes all show strong affinity for organic pollutants, in particular 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its related congeners. We undertook to use these compounds as a sorbent matrix in extracting TCDD from distilled water samples in the one to four parts-per-quadrillion (ppq) concentration range. Here we show the efficiency of these various sorbents with carbon nanotubes and activated carbon demonstrating near equal efficiency at the 1-4 ppq range, while C_{18} showed strong results only in the 60-80 ppq range. At 1 ppq activated carbon recovered 44.5% of the initial TCDD spike, while the carbon nanotubes demonstrated a recovery of 42%. It is our view that both activated carbon and nanotubes offer a means by which TCDD and related compounds may be removed from water samples as a necessary step toward environmental monitoring of natural water. While activated carbon and carbon nanotubes showed equal efficiency, carbon nanotubes may offer greater potential as a sorbent matrix based on the calculated high capture and potential for reuse.

Introduction:

The issue of water contamination due to dioxin is a seminal topic of debate both nationally and in the state of Maine. Dioxin is part of a large family of toxic substances that resist decomposition. These compounds are fat-soluble and accumulate in organisms from dietary sources. Polychlorinated dibenzo-p-dioxins (PCDDs) are manufacturing byproducts, which bioaccumulate even in areas devoid of human habitation due to atmospheric deposition. PCDD contamination of living organisms is complicated by their slow metabolism and excretion, which produces a spectrum of toxicological responses in animals.

Generally, PCDDs exist in extremely small quantities in the environment. This creates a number of problems because dioxin is a potent toxin even at very low concentrations. For this reason we have worked on a method for detecting PCDDs in water in the 1-4 parts-per-quadrillion (ppq) concentration range.

Historically, a number of different methods have been used to overcome the inherent problems of detecting dioxin in natural water. Here in Maine, fish sampling has been used for the past ten years. This method involves catching fish from suspected areas of contamination and then analyzing their tissues for dioxin. This method is both expensive and limited in its ability to produce statistically reliable data due to variation in fish population.

Another method currently under consideration is the use of Semi-Permeable Membrane Devices (SPMDs). SPMDs are submersible filtration devices that are anchored for a period of time in a waterway suspected of dioxin contamination. They are then collected and analyzed using mass spectroscopy. These devices show promise and have the potential to be far more reliable than current fish testing methods; but they are still hampered by high cost and the inherent difficulty in standardizing the effects of water temperature, biofouling due to microorganisms, and particulate matter, which is known to affect the capture rate of dioxin.

We have entered a joint venture with the Maine based company CAPE Technologies to modify immunoassay kits in order to provide an inexpensive alternative for dioxin monitoring in natural waters. CAPE Technologies has produced effective kits for monitoring dioxin, dibenzofurans, PCBs and dioxin-like PCBs in solid waste using immunoassays. Our partnership with CAPE Technologies has developed these kits for monitoring dioxin in natural water. These kits correlate the toxic equivalent concentration (TEQ) contributed by dibenzo-p-dioxin and dibenzo-p-furan. CAPE's immunoassay kits designed to test solid waste have been shown to have high sensitivity and high specificity for toxic PCDD/F congeners. These kits have already been used for determining PCDD/F levels in solid waste such as soil for several decades, and have been shown to have high affinity for the most toxic PCDD congener 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Our method has avoided these problems by offering a means of concentrating dioxin present in a relatively small sample size such as twenty liters. This method eliminates the inherent statistical limitations of fish sampling as well as the large volume sampling technique used with SPMDs. Here we use filtration columns with several types of sorbents to pass water through a column, which is then eluted and analyzed using immunoassay kits. The advantage of this method is that it is cost effective and avoids the inherent statistical limitations presented by other analytical techniques. Because our water sample is relatively small, we do not suffer from the complications of biofouling and water temperature fluctuations. Also because our method does not include biological sampling, we are not faced with the limitations of determining fish population migration.

Our project adapted current immunoassay kits produced by Cape Technologies of Maine for use in water sampling. In order for this product to function as a monitoring device for natural water, we developed a method to concentrate the dioxin present in the effluent. Our results indicate that this novel device will allow for detection of dioxin and dioxin-like compounds at 1ppq or less. Our research provides the necessary means for concentrating dioxin present in water samples. The growing concern for the quality of the natural water supply both in our state and the world will provide a broad market for this product serving both the economic and the environmental needs of the state of Maine.

Methods:

In order to overcome problems associated with testing such dilute concentrations of dioxin we chose to use several types of extraction columns. Our first choice was the

commercially available Solid Phase Extraction (SPE) column by Burdick and Jackson made from C_{18}. This collection column is approved for EPA method 525 and is made from a glass barrel column into which are packed eighteen molecule long carbon chains (C_{18}). The carbon is sandwiched between two small-pore frits.

The second type of column was prepared by taking 0.5 g of activated carbon and packing it into a glass barrel column similar in style to the Burdick and Jackson device. The activated carbon was again packed between two small-pore frits. These columns were constructed in our laboratory at the University of Maine.

Our third type of column was made from carbon nanotubes packed in a glass barrel column such as those previously used. Carbon nanotubes were supplied by Professor C. Rao of Jawaharial Nehru Centre for Advanced Scientific Research, India and from Professor L. Dai of the University of Australia. The carbon nanotubes (0.5g) were hand-packed and held in place by small-pore size frits. Carbon nanotubes are carbon molecules in the shape of a geodesic dome (C_{60}) that are woven together to form long tubes open at both ends. Previous researchers have found that carbon nanotubes have an extremely high capture rate for compounds such as dioxin and other organic contaminants[1].

All columns were washed prior to their being used as extraction devices. This procedure used 5 mL of hexane followed by 5 mL of isopropyl alcohol and finally 5 mL of

[1] Long, R., and Yang, R. *J. Am. Chem. Soc.* **2001**, 123, 2058.

deionized/distilled water. Columns were then allowed to air dry while attached to a vacuum for 10 minutes.

The Burdick and Jackson SPE C_{18} columns were tested at 80 ppq, 16 ppq, 4 ppq and 1 ppq concentration range. For the 80 ppq and 16 ppq samples 80 pg and 16 pg of TCDD respectively were added to 5 mL of isopropyl alcohol. These individual spikes were then added to 1 L of distilled water. For the 4 ppq sample, a total of 40 pg of TCDD was added to 10 mL of isopropyl alcohol and then added to 10 L of distilled water. Water samples were stirred for 3 minutes prior to extraction. For the 1 ppq sample, 20pg of TCDD were added to 20 mL of isopropyl alcohol and then mixed with 20 L of distilled water. Again samples were stirred for 3 minutes prior to extraction. All columns were run using the KNF liquid diaphragm pump as a vacuum source.

The activated carbon columns were tested at the 4 ppq and 1 ppq concentration ranges. These experiments followed the same protocol as noted above. The carbon nanotube columns were run in the 1 ppq range by adding 20 pg of TCDD to 20 mL of isopropyl alcohol and mixing with 20 L of distilled water. In every trial, procedural blanks were run to verify the accuracy of our results.

After filtration each column was eluted with 15 mL of toluene. This was done by pressurizing each column with a syringe after toluene had been added. The elute was captured in a disposable test tube and evaporated under nitrogen gas while standing in a heat bath. The samples were then analyzed using CAPE Technologies immunoassay kit.

Results:

We were successful at both 4 ppq and 1 ppq using activated carbon as a sorbent material. At the 4 ppq range we were able to recover 64% to 68% of the original TCDD spike. At the 1 ppq range we were able to recover 44% to 45% of the original TCDD spike. Results were positive for each concentration range, demonstrating that activated carbon was sufficient at removing small concentrations of TCDD from water samples.

We were also successful using the carbon nanotubes as a sorbent material. Results were positive at the 1 ppq range with recovery of approximately 42% of the original TCDD spike. Data was calculated using the Calculation Module C for DF1 for low to mid pg/g quantitative analysis supplied by Cape Technologies.

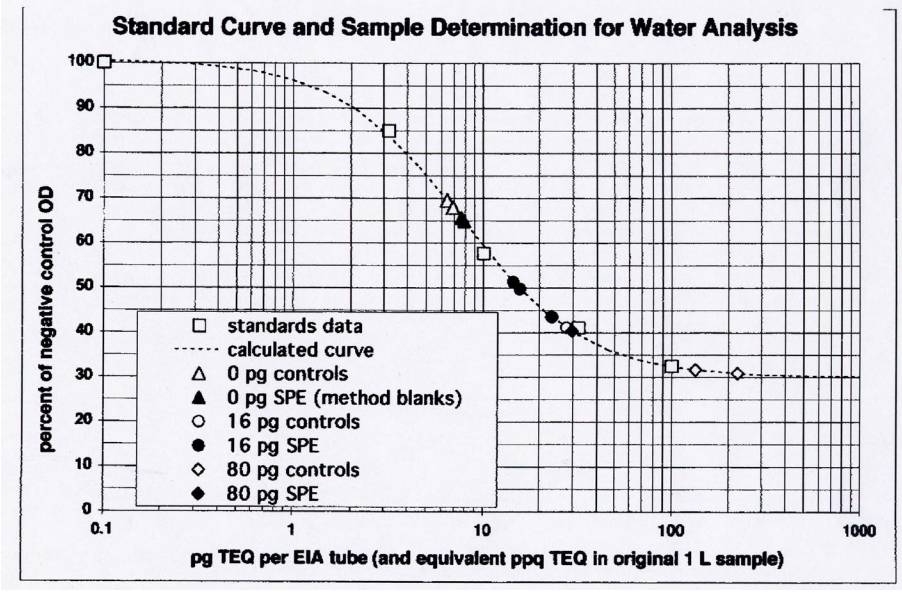

Figure 1. Data for initial tests of TCDD extraction from water samples. Distilled water was spiked with 16 pg of TCDD in 1 L of water (16 ppq) and also at 80 pg in 1 L of water (80 ppq). Data also shows two method blanks. This was calculated using the analytical module supplied by CAPE Technologies.

Figure 1 shows data from an initial test of the Burdick and Jackson SPE columns for capturing dioxin in water samples at moderate concentration levels. These tests were run using a single liter of water. The TCDD spike was first added to 5 mL of isopropyl alcohol in order to ensure the TCDD was completely dissolved. Capture rates varied from 70% to 81%. Immunoassay response curves are sigmoidal in shape. Equation 1 shows the four parameter equation used for fitting a calculated curve to the actual data. This ELISA test uses test tubes coated with anti-dioxin antibodies. The test sample is allowed to incubate in the tubes for twenty-four hours at which time a competitive conjugate is added. This competitor binds sites of the dioxin anti-body that have not already been occupied by dioxin molecules. A change in color is due to the amount of antibodies that are bound to the competitor. For this reason the CAPE Technologies immunoassay test develops a lighter chromographic response the more dioxin is present. The graph in figure 1 plots optical density (depth of color) against concentration. Samples containing higher amounts of dioxin are shown at the lower end of the curve. In other words, as the color of the tubes decreases the sample has a greater amount of dioxin.

$$Y=[(A-D)/(1+(X/C)^B)] +D \qquad \textbf{Equation 1}$$

Here: X= pg per immunoassay test tube (EIA); Y= normalized EIA response; A= Y value of upper asymptote; B= degree of curvature (negative slope of middle region); C= X value of midpoint curve; D= Y value of lower asymptote. These results were derived from the elution method previously described with the exception that dichloromethane was used in place of toluene. The immunoassay procedure was otherwise the same.

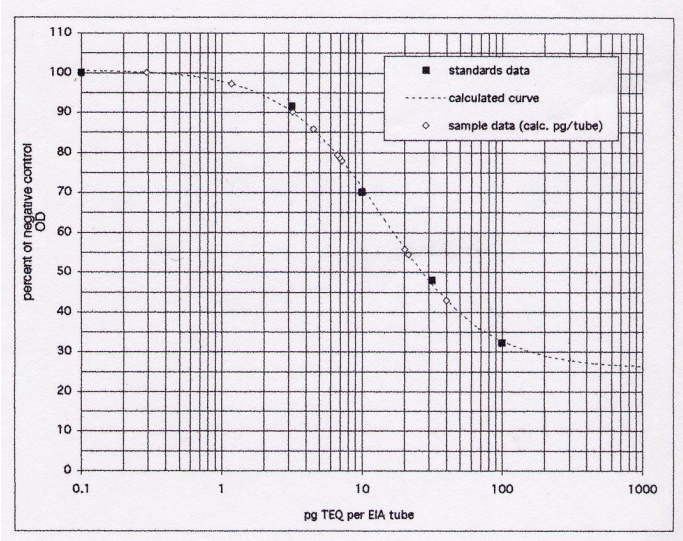

Figure 2. Data collected for water samples spiked with low concentrations of TCDD. Internal standards are represented by darkened squares, experimental data is represented by open triangles. This graph includes results of both the activated carbon and carbon nanotube columns at 1 ppq and 4 ppq.

Sample ID	*20 L x 1ppq (activated carbon)*	*20 L x 1ppq (activate carbon)*	*20 L blank*	*20 L blank*	*10 L x 4ppq (activated carbon)*	*10 L x 4 ppq (activated carbon)*	*20 L x 1ppq (nano-tubes)*	*20 L x 1ppq (nano-tubes)*
Calc. Pg/tube	7.3	7.0	0	0	21.8	20.4	40.3	6.7
Expected pg based on spike	16	16	0	0	32	32	16	16
Percent recovery	45%	44%	0	0	68%	64%	*252%	42%

Table 1. Data from the lower concentration tests (1-4 ppq). It should be noted that the first carbon nanotube sample produced a precipitate during the experiment. This data set is marked with a *. It is shown above, but was not used to calculated the average percentage of TCDD captured by carbon nanotubes.

Table 1 tabulates the data taken from figure 2. It is important to note that the data in row 3 corresponds to a specific loss of eluted sample due to the immunoassay's analytical procedure. Not all of the eluted sample may be recovered due to inherent limitations of pipetting. Consequently, a fixed 20% loss is calculated based on recovery techniques. Therefore, while the 4ppq samples contain 40 pg of TCDD, only 32 pg is delivered to the EIA tube. This is the same for the 1 ppq sample that theoretically has 20 pq of TCDD, where only 16 pg make it to the EIA tube.

There is also a discrepancy with one of the carbon nanotube samples. When running the test we noticed a yellow precipitate after the column was eluted. This impurity may derive from a number of sources. This sample is marked * and was not included in the averaged calculated recovery of TCDD from carbon nanotube columns.

Discussion:

All tests were able to recover enough TCDD to successfully analyze by immunoassay. At the 1 ppq range, the activated carbon recovered on average 7.2 pg of TCDD from a spiked sample containing 20 pg (adjustments for loss of TCDD due to pipetting limitations assumes a maximum of16 pg are present) for an average recovery of 44.5 %. At the 4 ppq range, the activated carbon recovered on average 21.6 pg from a spiked sample containing 40 pg of TCDD (again a 20 % loss of TCDD is included in the calculations). The average percent of recovery at this range was 66%. The discrepancy between the percent of recovery at the two different concentrations ranges may be due to a number of factors. It is inevitable that a portion of each TCDD sample is lost due to

glassware and other portions of the apparatus. As the concentration of TCDD decreases a greater proportion is lost by virtue of the glassware due to increased sample volumes. It is entirely possible that the greater amount of water washes some of the TCDD from the column during the filtration procedure.

The carbon nanotube column was successful at removing TCDD from water at the 1 ppq range. On average 6.7 pg of TCDD were recovered from a sample spiked with 20 pg (adjusted to 16 pg by previously described method) of TCDD for an average recovery of 42%. While carbon nanotubes have been previously shown to have a much higher affinity for TCDD compared to activated carbon, the discrepancy requires explanation. Because carbon nanotubes hold TCDD so tightly it is probable that these samples were not eluted with sufficient solvent to remove the TCDD trapped in the column. Future work must look at the solvent volume needed to completely remove any TCDD from the nanotube column. It may also be possible to heat toluene in order to increase the efficiency of elution. The advantage of the nanotube columns is their ability to capture TCDD so efficiently. With added work we should be able to develop a protocol by which the TCDD is completely freed from the nanotube column. This would allow us to use much smaller sample volumes and so greatly increase the efficiency of this method.

Our research has provided the necessary means for concentrating dioxin present in water samples. This provides a novel and reliable application for immunoassay kits. The growing concern for the quality of the natural water supply both in our state and the

world will provide a broad market for this product serving both the economic and the environmental needs of the state of Maine.

Summary:

We have successfully developed a method to remove dioxin from water samples at 1 ppq in sufficient quantities to perform analysis. For this purpose we have shown two sorbents to be of near equal efficiency. At the 1 ppq range activated carbon demonstrated a capture rate of 44.5 % compared to the carbon nanotubes with a capture rate of 44%.

It is our belief that the nanotubes offer greater future benefits compared to activated carbon. More needs to be done to examine the ability of the nanotubes to capture dioxin and dioxin-like toxins. Improved elution procedures will allow carbon nanotubes to attain greater capture rates compared with activated carbon and offer the additional benefit of being reusable.

This Seed Grant has allowed us develop a means of capturing dioxin at 1 ppq concentrations. These results allow for immunoassay tests to be conducted in field settings. Additional studies will have to address more complicated water samples, such as those containing particulate and organic matter. We have shown that it is possible to test natural water at 1 ppq dioxin concentrations. Further work will improve upon this method as a screening tool for dioxin in natural waters by environmental managers. In

conclusion, with this success we will seek further funds in order to develop a field tested method for screening TCDD in natural water using our concentration techniques.